Challenges in the Transfer of Complex Assay Formats

Cheryl McAlpine, Matt Bentley, David Shaw and Ray Briggs
Eurofins Pharma Bioanalysis Services UK Limited. Oxford, UK

Common Issues with Method Transfer

Method transfers can differ widely in scope but they all share the objective of needing to demonstrate acceptable assay performance at transfer site.

There are various reasons for an assay transfer that may include:

- Transfer from R&D to Regulatory environment
- Resource/ technology availability
- Cost & efficiency
- Accelerate study timelines
- Partner with technical expertise

An effective transfer strategy should be developed by originator and destination facilities to take account of the potential differences between sites, such as

- Equipment
- Readout technology
- Reagents
- Techniques
- Interpretation of methodology and/or data

The transfer of such a large amount of detailed information naturally requires effective communication. Communication requirements must be factored into the transfer strategy and monitored to ensure information is transferred in optimal formats and understanding is demonstrated. Communication is of primary importance particularly when transferring complex methods.
In this poster we will describe three case studies where complex assay procedures, involving use of cell lines, have been transferred into our laboratory and will highlight the principles and practices which can be most effectively employed to deliver an efficient method transfer, and will also highlight the challenges that need to be addressed and the pitfalls that should be avoided.

Pre-Transfer
- Early planning; sufficient time
- Understand needs
- Suitability of methods

Develop Effective Documentation
- Transfer study plan: 'What, How, When'
- Agreement between sites
- Detailed, tailored objectives
- Pre-determined acceptance criteria

Lab Phase
- Method optimised as required for purpose
- Continuous feedback
- In-line with recommendations

Reporting
- Final Report of complete study
- Capture solution based approaches
- Demonstrate 'fit for purpose'

Case Study 1
Neutralising Antibody Assay

Objectives of Transfer Study:
- Confirm suitability of assay for detection of neutralising antibodies to drug in human serum
- Confirm performance of assays as a pre-requisite for validation, including robustness

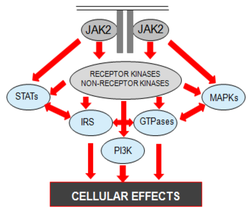
Target
- Hormone Receptor

Drug type
- Agonist

Mechanism of Action
- Multiple signalling pathways can mediate pleiotropic actions of ligand

Functional readout
- Cell Proliferation
- Convergence of many signalling outputs

Challenges & Solutions:
Ensuring 'Fit for Purpose'



Adapted from Zhu *et al* Cellular Signalling 2001; 13(9); 559

CRITERIA	Assay Transferred	Optimised Assay
Cell line / Target Receptor	Mouse Mutated /humanised rat	Human Full length human
Sub-culture required	Minimum 2 week	None
Stability of S/N	Day to day variability	Stable
Impact of sub-culture duration	Variability in sensitivity	Subculture not required Uniform cell 'age' / condition
Robustness	Inadequate (likely to cause failure of acceptance criteria to be met in SA)	Acceptance criteria meet for sensitivity & system suitability
Intended Use ▪Start up time ▪Planning Required ▪Data turnaround time	 Minimum 2 weeks Advanced- adequate flasks for no. plates needed Within 4 weeks	 Immediate Stock of vials in liquid N_2 Within 2 weeks

Conclusion
- By clearly establishing a priori criteria for the assay it was possible to come to a rapid agreement on a revised assay strategy.
- Trust and rapid decision making was enhanced by having evaluation criteria in place and effective communication forums for scientist to scientist discussion.

Case Study 2
PK Analysis

Objectives of Transfer Study:
- Confirm suitability of assay for pharmacokinetic analysis of clinical samples
- Confirm performance of assays as a pre-requisite for validation

Target
- Chemokine Receptor

Drug type
- Antibody

Functional readout
- Target binding

Assay Principal
CHO cells stably expressing target receptor incubated with samples to allow drug present to bind to cell surface target receptor. Antibody PE-conjugated detection reagent used to quantify drug present using calibration curve and data acquired by flow cytometry

CRITERIA	Transfer Assay	Optimised Assay
Analytical Procedure	Typographical errors in procedure	Method defined, errors corrected
Intra-assay / inter-assay precision	Highly variable CVs Suspected to be due to variable residual volumes	Method altered for plate addition, removing possibility of residual volume: Acceptance criteria met
Minimum Required Dilution (MRD) of Serum	In the presence of serum: Calibration curve altered Poor recovery reflected by high %RE Poor accuracy reflected by high %CV	Lowest concentration of serum selected that produced highest number of calibration points with acceptable %RE/%CV. Similar profile to assay buffer diluent
Calibration Curve	Profile of curve needs to be optimised to enable accurate reading of spiked QC samples	Recommended to increase number of points in calibration curve to extend linear portion of curve

Conclusion
- Critical for both parties to maintain an open dialogue throughout the transfer process, and avoid assumptions.
- Methods that are fit for purpose in a research environment likely not be fit for regulatory studies.
- Clear, defined criteria for method performance again helped to get rapidly to the right solution.
- As part of transfer study optimise the method in discussion with the origin lab, such that it can become adequate for its intended use in regulatory studies

Case Study 3
Drug Potency Assay

Objectives of Transfer Study:
- Confirm suitability of assay for measurement of drug potency
- Assess feasibility of reliable use for batch release/ stability studies

Target
- Interferon

Drug type
- Antibody

Mechanism of Action
- Inhibition of signalling protein up-regulation

Functional readout
- Luciferase reporter gene

Assay Principal
Engineered HeLa cells harbouring a luciferase reporter under control of a promoter induced upon drug binding to target, maintained in subculture and seeded in 96 well plate prior to use in assay. After administration of drug, a luminescent luciferase assay was used to calculate potency.

Challenges & Solutions:
Ensuring 'Fit for Purpose'

CRITERIA	Assay Transferred	Optimised Assay
Sensitivity	Instability in assay signal leading to high day to day variability.	Optimal growth conditions and plate seeding density established: Maximising S/N Reproducible sensitivity profile
Robustness	Luminescence readout unstable, with signal reduction both across the reading plate and over time	Alternative detection reagent with greater half-life introduced: Improved robustness Signal was reduced but fit for purpose
Precision	Sub-optimal assay signal particularly at lower drug concentrations contributing to high %CV.	Replaced use of less sensitive multi functional plate reader with dedicated luminometer Increased sensitivity Improved precision Extended dynamic range

Conclusion
- Once again a clear position on the necessary performance criteria provided a firm basis for a rapid, agreed, solution-based response to problems encountered.

Summary

Method transfer is an opportunity for (re)evaluating the assay and generating valuable information that could lead to further optimization of robustness and workflow. Two principle components of effective method transfer are:

***A priori* acceptance criteria**
- To facilitate like for like evaluation of the method at both originator and destination laboratories
- Enable identification of areas for improving the method

Communication plan
- Agreed schedule of regular forums for destination lab scientists to update originator labs on progress, issues and suggestions for method improvement as required